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Assessment of serum ionic profiles in rabbits treated by *Mitracarpus scaber* (Rubiaceae).

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ABSTRACT

This study aims to investigate the ionic disturbs of *Mitracarpus scaber* (Rubiaceae) in rabbits. It is a plant traditionally used to treat skin diseases and various ailments in Côte d'Ivoire and elsewhere in West Africa. For this study, different batches of rabbits were injected with increasing doses of aqueous extract of *Mitracarpus scaber* (encoded Misca). Then changes in serum calcium, magnesium, chloride, sodium and potassium were evaluated. According to statistical analysis of results, the use of the aqueous extract of *Mitracarpus scaber* with doses between 12.5 and 200 mg / kg body weight (bw) in rabbits causes a significant variation ($P < 0.05$) of calcium serum concentrations. But there is no significant change ($P > 0.05$) of magnesium, chloride, sodium and potassium serum concentration. This study suggests that a reduction of the dose (100 mg / kg) and time of treatment (4 weeks) may help to avoid ionic disturbs other time. This dose of 100 mg /kg/bw which is much higher than the therapeutic dose, confer on *Mitracarpus scaber* a safety margin 530 (Tolerate Maximum Dose/ Therapeutic dose) very interesting.

Keywords: *Mitracarpus scaber*, calcium, magnesium, chloride, sodium, potassium

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INTRODUCTION

Skin diseases occupy a prominent place in Côte d'Ivoire, as in all countries of the tropical zone. Dermatitis remains a real public health problem and stands for the third reason of consultation in rural areas. Their management is made difficult by the inaccessibility to health care and the low number of dermatologists [1]. This situation obliged people to resort more and more to medicinal plants that are easily accessible [2]. This is the case of *Mitracarpus scaber* (Rubiaceae), a plant traditionally used in Côte d'Ivoire and elsewhere in Africa to treat sores, ringworm and various diseases.

The antibacterial and antifungal activities of *Mitracarpus scaber* (encoded MISCA) have been highlighted by several studies [3-7]. It has a marked activity on 12 germs among which we can quote: *Aspergillus, Cryptococcus, Trichophyton, Candida, Staphylococcus, E. coli,* which are opportunistic pathogens of AIDS. Minimum Fungicidal Concentration (MFC) of MISCA is 0.20 mg / ml while IC_{50} is 0.10 mg / ml [8-10]. In addition to dermatitis, it should be noted that the work of Aboughe helped to highlight the cardiodepressent effect in rats [11].

Given the excellent results of pharmacological tests and the wide use of this plant, a rationalization of its use is required. Especially in view of its potential use in cardiovascular therapeutics. Indeed, the therapeutic use of plants is not always without danger to the user populations [12, 13]. These plants can lead important ionic disturbs as well as many drugs. Hydromineral imbalances that are induced by several drugs can have serious consequences such as latent metabolic acidosis that directly affects the transport of oxygen and cell nutrition. Decreased enzymatic activity, impairment of the immune system constituting a ground conducive to the emergence of many diseases such as: the risks of osteoporosis, cardiac, metabolic and thyroid disorders [14-16]. Acute and subacute toxicity of MISCA in the Swiss mouse have been evaluated. The results made it possible to obtain the toxicological parameters such as the lethal dose for 50% (LD_{50}= 515 mg/kg of body weight), the lethal dose for 100% (LD_{100} = 800 mg/kg bw) while the maximum tolerated dose (MTD) of the aqueous extract of MISCA is 200 mg / kg of body weight [17].

In the logical continuation of this work, we wanted to deepen the state of knowledge on bio-tolerance of MISCA during this study. More specifically, it is to assess the ionic disturbs of the aqueous extract of MISCA following changes of many specific ions in rabbits: calcium, magnesium, chloride, sodium and potassium. Serum variations in these parameters can thus assess the impact of this extract on ionic disturbs.

MATERIAL AND METHODS

Experimental

Plant material

The leaves of *Mitracarpus scaber* (Rubiaceae) collected from peripheral areas of Abidjan (Côte d'Ivoire) were identified by the National Floristic Center of University of Cocody-Abidjan. A voucher specimen (N° 13612) of the plant has been deposited in this Center herbarium.

Experimental animals

Rabbits, *Oryctolagus cuniculus* (36) of 8-10 weeks old, weighing 1.17±0.22 kg and bred at the Department of Biosciences, University Felix Houphouet Boigny (Abidjan, Ivory Coast), were used for the experiments. They come from a rabbit cattle farm in Bingerville (Ivory Coast). The animals were kept in standard cages with good ventilation, free access to food and water. Experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences of University Felix Houphouet Boigny (Ivory Coast -Abidjan). These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals [18].

Preparation of aqueous extract of *Mitracarpus scaber* (Rubiaceae)

Plants harvested were air dried at room temperature (28±1 °C) for one month. The dried leaves were ground into fine powder. The powder (100 g) was soaked in two liters of distilled water for 48 hours on a

magnetic agitator (IKAMAG RCT). The extract was filtered twice through cotton wool, and then through Whatman filter paper (3 MM). The filtrate was evaporated to dryness in a rotary evaporator (BUCHI) at 60 °C. After drying, we get a greenish powder used to prepare the aqueous extract of MISCA.

Experimental protocol

After randomization into 6 groups of 6 rabbits (3 males and 3 females), and before initiation of experiments, the rabbits were acclimatized for a period of 14 days under standard environmental conditions of temperature, relative humidity, and 12 h dark/light cycle. Animals had free access to food and water *ad libitum*.

Animals in each group were separated according to their sex in cages. Among these 6 groups, five experimental groups have received doses ranging from 12.5 to 200 mg/kg of bw (which is the Maximum Tolerated Dose (MTD) of the aqueous extract) in a geometric progression of ratio two [17]. Twice a week for six weeks, the animals received intraperitoneally 0.2 mL of an injection according to their group. Each rabbit of batch 1 (control) received only 0.2 mL of physiological solution of 0.09% Nacl (B. Braun) used to administrate extracts. Rabbits of batch 2 to batch 6 received respectively 12.5; 25; 50; 100 and 200 mg/kg of bw.

Blood samples were collected in the morning (from 8 to 11 h) via the marginal ear vein of the animals, once a week using sampling needles. Blood sampling was carried out once a week in the one week preceding the first application of treatment (w_0), during the five weeks of treatment (w_1, w_2, w_3, w_4, w_5 and w_6). These blood samples were collected in sterile tubes without anticoagulant. There were centrifuged at 3000 rpm for 10 min using a liquidizer JOUAN. Serum ions were measured with an automatic analyzer, LIASIS while sodium and potassium have been measured with spectrophotometer flamme SEAC *fp* 20.

Assay for ions (calcium, magnesium, phosphorus, chloride)

The principles of the determination of each parameter are described according to the manufacturer's instructions reagents.

Calcium (Spinreact): The measurement of calcium in the sample is based on formation of color complex between calcium and *o*-cresolphtalein in alkaline medium. The intensity of the color that is proportional to the calcium concentration in the sample is measured in a spectrophotometer at 570 nm wavelength

Magnesium (Spinreact): Magnesium forms a purple colored complex when reacts with calmagite in alkaline solution. The intensity of the color formed that is proportional to the magnesium concentration is measured in a spectrophotometer at 520 nm wavelength.

Chloride (Spinreact): The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate and subsequent formation of a red ferric thiocyanate complex is measured colorimetrically. The intensity of the color formed which is proportional to the chloride ion concentration in the sample is measured in a spectrophotometer at 480 nm wavelength.

Sodium and **potassium** have been measured with spectrophotometer flame SEAC *fp* 20.

Statistical Analysis

The data were processed using the software Graph Pad Prism 5.0 (Microsoft, USA). The analysis of variance (ANOVA) was performed according to the multiple comparison test of Tukey for the comparison of mean values of serum ions of different groups but also to relative baseline in each group. Data are presented means ± standard error of mean (S. E.M) for the number of animals in each group (n = 6). The difference is said to be significant if ($P<0.05$) and not significant if ($P>0.05$).

RESULTS

The results of changes in serum, calcium, magnesium, chloride, sodium and potassium expressed in tables (1, 2, 3, 4 and 5) are averages of six assays performed in each group.

Calcium:

The serum calcium (w_0) was 90.6±1.03 mg/L in the untreated lot (lot1). This value varies over time between 89.17±7.7 mg/L (minimum w_2) and 91.67±3.1 mg/L (maximum w_4), representing a change of -1.65% (w_2) to 1.10% (w_4, w_5) of the initial rate of serum calcium**.** In lot 2 (12.5 mg / kg), serum calcium was 91.33±1.6 mg/L before treatment. Over the past six weeks, the rate changes of 90.5±5.54 mg/L (minimum w_2) to 91.33±1.6 (maximum w_1). These values correspond to variations of -0.91% (w_2) to 0.54% (w_1) (Table 1).

In group 3, serum calcium rate was 90.6±1.36 mg/L before treatment. This value varied to 85±4.47 (minimum w_2) to 92.83±3.2 mg/L (maximum w_5). These evolutions represent variations of -6.25% (w_2) to 2.39% (w_5).

Percentage changes as recorded in lots 4, 5 and 6 are respectively: -3.65% (w_1,w_2) to 1.85% (w_6); -0.37% (w_3) to 2.94% (w_1) and 2.05 % (w_2) to 8.76% (w_6).

Statistical analysis of the results indicates a significant change in serum calcium ($P < 0.05$), especially with the dose of 200 mg / kg bw (lot 6) in the fifth and sixth week.

Magnesium:

The serum magnesium (w_0) was 18.8±0.98 mg/L in the untreated group (group 1). This value which varies over time from 17.5±2.59 (minimum w_6) to 20±3.9 mg/L (maximum w_2), represents a variation of -7.07 % (w_6) to 6.19 % (w_2) of the initial serum magnesium. In lot 2 (12.5 mg / kg), serum magnesium was 19±1.26 mg/L before treatment. Over the past six weeks, the rate changed of 18±0.89 (minimum w_3) to 19±1.55 mg/L (maximum w_6). These values correspond to variations of -5.26% (w_3) to 0% (w_6) (Table 2).

Serum magnesium rate in batch 3 was 19±1.54 mg/L during the week before treatment (**w_0**). This value changed from 17.8±0.7 (minimum w_5) to 19±0.9 mg/L (maximum w_4). These variations represent -6.14% (w_5) to 0% (w_4).

Percentage changes as recorded in batches 4, 5 and 6 are respectively: -5.56% (w_4) to 5.55% (w_5); -6.54% (w_4) to 1.86% (w_6) and - 5.55% (w_4) to 5.55% (w_5, w_6) of the initial serum magnesium.

The statistical analysis shows no significant change in serum magnesium with different doses ($P > 0.05$).

Chloride:

The serum chloride at w_0 was 99.33±1.97 mEq/L in the untreated lot**.** This value which varies over time between 98.33±1.86 mEq/L (minimum w_1) and 104±3.22 mEq/L (maximum w_6), represents a variation of -1.00% (w_1) to 4.69% (w_6) of the initial rate of chloride. In batch 2 (12.5 mg / kg), serum chloride was 98.83±1.72 mEq/L before treatment. Over the past six weeks, the rate changed from 98±2.28 mEq/L (minimum w_4) to 101.7±0.82 mEq/L (maximum w_6). These values correspond to variations of -0.84% (w_4) to 2.86% (w_6) of the initial serum chloride (Table 3).

Before treatment, serum chloride rate was 98.67±2.25 mEq/L in lot 3. This value varied from 97.67±0.5 (minimum w_3) to 101.3±1.03 mEq/L (maximum w_6). These variations correspond to -1.01% (w_3) to 2.70% (w_6). In group 4, chloride serum rate was 100±0.89 mEq/L during w_0. The percentage change during the weeks of treatment is -0.33% (w_4) to 1.67%(w_6).

The percentage changes so recorded in batches 5 and 6 are respectively -1.34% (w_4) to 3.02% (w_5) and -0.67% (w_2) to 4.02% (w_6).

The statistical analysis shows no significant change in serum chloride with different doses ($P > 0.05$).

Sodium:

The serum sodium at w_0 was 139.3±2.42 mEq/L in the untreated lot**.** This value which varies over time between 134.2±4.58 mEq/L (minimum w_6) and 142.7±3.14 mEq/L (maximum w_3), represents a variation of -3.70% (w_6) to 2.39% (w_3) of the initial rate of sodium. In batch 2 (12.5 mg / kg), serum sodium was 141.3±1.97 mEq/L before treatment. Over the past six weeks, the rate changed from 140.2±1.83 mEq/L (minimum w_2, w_3) to 142.2±1.17 mEq/L (maximum w_6). These values correspond to variations of -0.82% (w_2, w_3) to 0.59% (w_6) of the initial serum sodium (Table 4).

Before treatment, serum sodium rate was 140±0.89 mEq/L in lot 3. This value varied to 141.3±1.03 mEq/L (maximum w_6). These variations correspond to 0% (w_3) to 0.95% (w_6). In group 4, serum sodium rate was140±1.79 mEq/L during w_0. The percentage change during the 6 weeks of treatment is -0.23% (w_1) to 1.19% (w_5).

The percentage changes so recorded in batches 5 and 6 are respectively -1.23% (w_1) to 2.37% (w_5) and -1.06% (w_2) to 0.82% (w_5).

The statistical analysis shows no significant change in serum sodium with different doses ($P > 0.05$).

Potassium:

The serum potassium at w_0 was 3.8±0.24 mEq/L in the untreated lot**.** This value which varies over time between 3.73±0.19 mEq/L (minimum w_1) and 4.4±0.32 mEq/L (maximum w_6), represents a variation of -1.75% (w_1) to 15.78% (w_6) of the initial rate of potassium. In batch 2 (12.5 mg / kg), serum potassium was 3.78±0.34 mEq/L before treatment. Over the past six weeks, the rate changed from 3.86±0.28 mEq/L (minimum w_1) to 4.33±0.23 mEq/L (maximum w_4). These values correspond to variations of 2.20 % (w_1) to 12.33% (w_4) of the initial serum potassium (Table 5).

Before treatment, serum potassium rate was 3.93±0.27 mEq/L in lot 3. This value varied from 3.9±0.15 (minimum w_1) to 4.36±0.33 mEq/L (maximum w_6). These variations represent -0.85 % (w_1) to 11.02 % (w_6).
In group 4, serum potassium rate was 3.96±0.19 mEq/L during w_0. The percentage change during the 6 weeks of treatment is -1.68 % (w_3) to 7.56% (w_4). The percentage changes so recorded in batches 5 and 6 are respectively 0 % (w_3) to 7.56 % (w_5, w_6) and -0.82 % (w_1) to 7.43% (w_5).

The statistical analysis shows no significant change in serum sodium with different doses ($P > 0.05$).

DISCUSSION

Variations in serum activities of enzymes stored in different batches before treatment and those recorded in the control group (batch 1) which has not undergone any treatment are in conformity with the usual values obtained in rabbits [19].

Statistical analysis of the results indicate that the aqueous extract of MISCA with the doses between 0 and 200 mg / kg body weight for six weeks, don't lead a significant change in serum magnesium, phosphorus, chloride, sodium and potassium. But there is a significant change in serum calcium. These variations are more pronounced with the dose of 200 mg/kg/body weight especially during the fifth and sixth week.

Calcium is substantially removed from the blood by glomerular filtration. The concentrations of these metabolites in urine are regulated by the kidney which has a real role of blood filter. It is also established that the glomerular filtration rate is dependent on the pressure in the glomerular capillaries about 30 mm Hg. The decline in blood pressure can cause a decrease in glomerular pressure about 10 mmHg. Any decrease in blood pressure may decrease plasma volume filtered by the kidney. Thus, ionic concentrations which are not correctly eliminated increase in the blood. That is here the case of calcium. This is one of the leading causes of kidney failure [20-22].

In fact, the link between changes in blood pressure and the occurrence of renal failure have been revealed by many authors [23-25]. This phenomenon has been described with other plants such as *Phyllanthus amarus* (Euphorbiaceae) and *Mareya micrantha* (Euphorbiaceae) [26, 27]. This could therefore suggest an induction of renal dysfunction with very high doses of the aqueous extract of MISCA. Indeed, MISCA would have a cardiodepressant activity on isolated rat heart coupled with an hypotensive effect on blood pressure at 4.5 mg / L.

In addition, the metabolism of several well-known calcium antagonists such as nifedipine and verapamil indicate that the kidney plays an important role in eliminating them. For example, 70-80 % of nifedipine is excreted by the kidneys, more than 90% of this amount is recovered in the urine after 24 hours, while the metabolites of verapamil, are excreted exclusively via the kidney for 70% [28-31]. These data confirm to wish that the kidney may play a key role in the elimination of the aqueous extract of *Mitracarpus scaber* like that of some calcium antagonists.

CONCLUSION

At the end of this work, it appears that the use of aqueous extract of *Mitracarpus scaber* at doses between 12.5 and 200 mg / kg of body weight in rabbits causes a non significant change in serum, magnesium, chloride, sodium and potassium, but a significant change in serum calcium. This increase is noticeable during the two last weeks with the dose of 200 m / kg bw. The high doses of *Mitracarpus scaber* (more than 100 mg / kg bw) could lead ionic disturbs. This study suggests that a reduction of the dose (100 mg / kg bw) and time of treatment (4 weeks) may help to avoid ionic disturbs in the long term. We note that with this dose of 100 mg / kg (106 mg / ml) which is much higher than the therapeutic dose (CMF= 0,2 mg/ml), *Mitracarpus scaber* always keep a safety margin of 530 very interesting.

However, it is necessary that the traditional use of this plant in decoction to relieve various ailments must be rationalized. Moreover, in order to better understand all aspects of bio-tolerance, it would be necessary to carry out further studies including cardiovascular and liver tolerance as well as, urinary metabolites and hematological investigations. Finally, the impact on parathyroid hormone which regulates phosphocalcic metabolism deserves to be evaluated carefully.

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CONFLICT OF INTERESTS: The authors claim that there is no conflict of interest.

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